	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments
1	BRS	L1	6194	prostat$3 near3 cancer	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:45	
2	BRS	L2	4931	HER2 OR HER-2 OR EBRB2 OR ERBB-2 OR C-ERBB-2 OR CERBB2 OR C-ERBB2 OR CERBB-2 OR P185 OR NEU OR 2C4	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:48	
3	BRS	L3	65	1 same 2	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:49	
4	BRS	L4	14	1 with 2	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:50	

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments
5	BRS	L5	502	2 near10 (antibod$3 or mab or ab)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:53	
6	BRS	L6	6	5 same 1	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:54	
7	BRS	L7	18366	prostat$3	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:54	
8	BRS	L8	11	5 same 7	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 13:54	

(FILE 'HOME' ENTERED AT 13:41:35 ON 29 JAN 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS, CANCERLIT, SCISEARCH' ENTERED AT 13:42:09 ON 29 JAN 2002

```
L1        124952 S PROSTAT### (3A) CANCER
L2         36994 S HER2 OR HER-2 OR EBRB2 OR ERBB-2 OR C-ERBB-2 OR CERBB2 OR
C-E
L3           570 S L1 (P) L2
L4          6078 S L2 (30A) (ANTIBOD### OR MAB OR AB)
L5           146 S L1 (P) L4
L6            48 DUP REM L5 (98 DUPLICATES REMOVED)
L7        307431 S PROSTAT###
L8           226 S L7 (P) L4
L9            80 S L8 NOT L5
L10           27 DUP REM L9 (53 DUPLICATES REMOVED)
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     (FILE 'HOME' ENTERED AT 16:22:07 ON 29 JAN 2002)

     FILE 'REGISTRY' ENTERED AT 16:22:15 ON 29 JAN 2002
                E MDX-H210/CN
L1              1 S E2

     FILE 'REGISTRY' ENTERED AT 16:23:31 ON 29 JAN 2002
L2              1 S 337308-14-2/RN
                  SET NOTICE 1 DISPLAY
                  SET NOTICE LOGIN DISPLAY

     FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS, CANCERLIT, SCISEARCH' ENTERED AT
     16:24:11 ON 29 JAN 2002
L3             82 S MDX-H210 OR MDX-H 210 OR MDXH210
L4             32 DUP REM L3 (50 DUPLICATES REMOVED)
L5             12 S L4 AND PROSTAT###
```

L6 ANSWER 36 OF 48 CANCERLIT
AN 96605241 CANCERLIT
DN 96605241
TI Role of c-erbB gene family in prostate cancer.
AU Ching K Z
CS Univ. of Manitoba, Canada.
SO Diss Abstr Int [B], (1995). Vol. 55, No. 11, pp. 4738.
ISSN: 0419-4217.
DT (THESIS)
FS ICDB
LA English
EM 199605
AB . . . the prostate and maintenance of adult structure and function, as well as its pathological disturbances, benign prostatic hyperplasia (BPH) and **prostate cancer**, are strongly influenced by testicular androgens. Unfortunately, present therapeutic approaches for **prostate cancer** which achieve androgen ablation are unsatisfactory. Such treatment frequently yields an initial beneficial response; however, nearly all patients eventually relapse to a hormone-insensitive state and succumb to progression of the disease. Our understanding of the continuing growth of **prostate cancer** cells in the absence of androgens is incomplete. Recent efforts have investigated the hypothesis that polypeptide growth factors and their. . . this important clinical observation. This thesis is focused on the contribution of the erbB gene family to the control of **prostate cancer** cell growth. Two complementary approaches have been used: first, the examination of human prostate tumors for erbB gene expression or perturbation, and second, the experimental manipulation of human **prostate cancer** cell lines to study erbB-mediated growth control. Elevated expression of the erbB 1 gene, the epidermal growth factor (EGF) receptor, and its ligands, EGF and transforming growth factor alpha (TGF-alpha) was demonstrated in prostate tumor samples and human **prostate cancer** cell lines. In two cell lines, Du145 and LNCaP, exogenous EGF and TGF-alpha stimulated cell growth, while antibodies to the. . . result indicates that mechanisms other than androgen- or EGF receptor-mediated growth are present in PC-3 cells. Overexpression of the erbB2 (**NEU**) gene also was demonstrated in prostate tumor samples and cell lines, and was associated in many instances with gene amplification. In the cell lines, monoclonal **antibodies** to the **c-erbB2/NEU** receptor inhibited proliferation and caused a decrease in the mRNA for the nuclear transcription factor c-fos. In LNCaP cells, a. . . of androgen or estrogen, both apparently acting through the mutated androgen receptor. However, c-erbB2/NEU expression was evident in all three **prostate cancer** cell lines in the absence of steroid hormones. These data confirm the involvement of the erbB gene family in regulating **prostate cancer** cell growth. Targeting the EGF receptor and the c-erbB2/NEU receptor may provide new avenues to inhibit **prostate cancer** growth in the clinical setting. (Full text available from University Microfilms International, Ann Arbor, MI, as Order No. AADAA-INN92134)

FILE 'HOME' ENTERED AT 12:20:32 ON 29 JAN 2002)

FILE 'REGISTRY' ENTERED AT 12:21:07 ON 29 JAN 2002
E 2C4/CN

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS, CANCERLIT, SCISEARCH' ENTERED AT 12:22:10 ON 29 JAN 2002

L1	307431	S	PROSTAT###
L2	338	S	2C4
L3	14	S	L1 (P) L2
L4	4	DUP	REM L3 (10 DUPLICATES REMOVED)
L5	89	S	L2 (3A) ANTIBOD###
L6	26	DUP	REM L5 (63 DUPLICATES REMOVED)

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
1	BRS	L1	18366	prostat$3	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 12:21			0
2	BRS	L2	173	2c4	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 12:22			0
3	BRS	L3	12	1 same 2	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 12:25			0

	Type	L #	Hits	Search Text	DBs	Time Stamp	Comments	Error Definition	Errors
4	BRS	L4	13	2 near3 antibod$4	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2002/01/29 12:26			0

STIC-ILL

RC254.J75 1

380658

From: Hunt, Jennifer
Sent: Tuesday, January 29, 2002 4:30 PM
To: STIC-ILL
Subject: Refernces for 09/602,800

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British Journal of Cancer, (1998) Vol. 78, No. SUPPL. 2, pp. 19

INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY, BIOLOGY, PHYSICS, (1998 Nov 1) 42 (4) 817-22

J. Urol. (Baltimore) (1999), 161(3), 984-989

Diss Abstr Int [B], (1995). Vol. 55, No. 11, pp. 4738

CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 210-5

Proc Annu Meet Am Soc Clin Oncol, (1997). Vol. 16, pp. A154

CLINICAL CANCER RESEARCH, (1998 Feb) 4 (2) 295-302

Proc Annu Meet Am Soc Clin Oncol, (1998). Vol. 17, pp. A1681

Proc Annu Meet Am Soc Clin Oncol, (1999). Vol. 18, pp. A1197

BRITISH JOURNAL OF CANCER, (SUM 1998) Vol. 78, Supp. [2], pp. 56-56

EUROPEAN JOURNAL OF CANCER, (SEP 1999) Vol. 35, Supp. [4], pp. 1388

IDrugs, (1999) 2/7 (624-628)

Thanks,

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C1STI
1/31

58 men with clinically localized
y on 4 RTOG phase III randomized
evaluable for this analysis. None
y with their initial treatment. All
ical review for grading using the
an follow-up time was 7 years. The

leason score was an independent
sease-specific survival (DSS) rates
4: 99% and 91%; 5–6: 95% and
and 43%. Stratification of men by
er radiation dose (≥ 66 Gy) was a
rol, DSS, and overall survival rates
0 ($p < 0.05$). After adjusting for
her radiation dose was associated
om prostate cancer ($p = 0.039$).
nificant effect for higher radiation
from prostate cancer in men with
his prognostic factor should be the
uating radiation dose escalation.
TOG U10 CA21661, CCOP U10
NCI.

were report in the *Journal of Clinical Oncology* in 1997.

Results: The following table reflects the findings of the study after a median follow up of 5.6 years for all patients and 6.0 years for living patients;

	Arm	Number Failures	Five Years	Eight Years	p Value
Local Failure	I	92	15%	23%	$p < .0001$
	II	155	31%	37%	
Distant Metastasis	I	103	15%	27%	$p < .0001$
	II	154	29%	37%	
NED Survival	I	244	44%	25%	$p < .0001$
	II	306	62%	36%	
NED Survival with PSA ≤ 1.5mg/ml	I	252	54%	32%	$p < .0001$
	II	370	21%	8%	
Cause-Specific Failure	I	65	9%	16%	$p = 0.23$
	II	80	13%	21%	
Absolute Survival	I	182	75%	49%	$p = .36$
	II	207	71%	47%	

A subset analysis of centrally reviewed Gleason 8–10 patients without prostatectomy showed that for patients receiving radiation therapy plus adjuvant hormones there is a statistically significant improvement in both absolute survival ($p = 0.036$) and cause-specific failure ($p = 0.019$).

Conclusion: Use of long term adjuvant androgen deprivation in addition to definitive radiation therapy results in a highly significant improvement with regards to local control, freedom from distant metastasis, and biochemical disease free survival in unfavorable prognosis patients with carcinoma of the prostate.

ons of Brachytherapy for Early Pros-
s. *James A. Talcott, Jack C. Clark,*
e. Medicine, Dana-Farber/Partners
r Health Quality, Outcomes, and
gers Memorial Veterans Hospital,
ncer Foundation, Northwest Hospi-

, or brachytherapy, have become an
prostate cancer. Long-term patient-
rted. To estimate long-term bowel,
omplication rates of this modality,
ated from 2 years 9 months to 10
ier assessments of conventionally-
ent by registered mail to randomly
therapy at Northwest Hospital in
f 167 questionnaires mailed, 131
5 patients (80%) of those returned
ing patients, 72 received brachy-
d both implants and external beam
and time since implant in both
ectively.
ncreased incontinence (85% leaked
13 men. Subsequent TURP and
ented. We conclude that long-term
r implants only, but that erectile
ary incontinence are both common.
ntify pretreatment status, require

eeds/External Beam (% of pts)	All patients (% of pts)
16	9
16	15
16	11
58	49
24	16
12	16
61	49
70	69

***1197**

Immunotherapy with the Bispecific Antibody MDX-H210 (anti-HER2 × anti-CD64) Combined with GM-CSF in HER2 Positive Hormone Resistant Prostatic Cancer. *ND James, PJ Atherton, AJ Howie, S Tchekmedyian, RT Curnow. CRC, Institute for Cancer Studies, University of Birmingham, Birmingham B15 2TA. Department of Pathology, University of Birmingham, Birmingham B15 2TT. Pacific Shores Medical Group, Long Beach CA. Medarex Inc. New Jersey, USA.*

Background: Prostatic cancer is the second commonest malignancy in men in developed countries and the second highest cause of cancer death. Treatment of hormone resistant cancer is a palliative in nature and new therapies are urgently needed. We report results following treatment with the bispecific antibody MDX-H210 (anti-HER2 × anti-CD64) combined with GM-CSF in patients with HER2 positive hormone refractory prostate cancer.

Methods: Patients were treated with GM-CSF 5 μg/kg/day by subcutaneous injection for 4 days plus MDX-H210 15 mg/m^2 by intravenous infusion on day 4, repeated weekly for 6 weeks. Patients continued on treatment without interruption until disease progression or unacceptable toxicity.

Results: 25 patients entered the trial, 1 received no treatment and 20 are assessable for response. Toxicity was generally NCI-CTG 0–2; there were 2 grade 4 adverse events (spinal cord compression, probably related to disease progression; nausea and vomiting). There were no deaths on treatment. 7 patients of 20 (35%) evaluable patients had a PSA reduction of >50%, ranging from 51% to 99%, of duration 71, 85, 92, 125, 131, 198+, and 215+ days. A further 6 patients experienced PSA reductions <50%, >25% of 87, 92+, 130, 134, 150 and 162 days duration. 4 of 15 (27%) patients with evaluable pain had improvements in pain scores. The PSA relative velocity (PSARV) on therapy was compared to the period prior to study entry and decreased in 16/18 (89%) patients with pre-study PSA data (paired t-test for pre-treatment vs. Post treatment PSARV, P[two-tailed] = 0.0006, N = 18). Median duration of follow up is 105+ days (range 21–215+ days).

Conclusions: The combination of GM-CSF and MDX-H210 is active in hormone refractory prostate carcinoma. Toxicity was generally mild to moderate and mostly manageable on an outpatient basis. Further studies in prostate cancer are indicated.

RC261.C61

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CANCER IMMUNOLOGY, IMMUNOTHERAPY, (1997 Nov-Dec) 45 (3-4) 210-5

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BRITISH JOURNAL OF CANCER, (SUM 1998) Vol. 78, Supp. [2], pp. 56-56

EUROPEAN JOURNAL OF CANCER, (SEP 1999) Vol. 35, Supp. [4], pp. 1388

IDrugs, (1999) 2/7 (624-628)

Thanks,

Jennifer Hunt
Patent Examiner, Art Unit 1642
CM1-8D06 (mailbox 8E12)
(703)308-7548

Expression of Potential Target Antigens for Immunotherapy on Primary and Metastatic Prostate Cancers[1]

Shengle Zhang, Helen S. Zhang, Victor E. Reuter, Susan F. Slovin, Howard I. Scher, and Philip O. Livingston[2]

Clinical Immunology Service [S. Z., H. S. Z., P. O. L.] and Genitourinary Oncology Service [S. F. S., H. I. S.], Department of Medicine, and Department of Pathology [V. E. R.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021

ABSTRACT

Defining the expression of tumor-associated antigens on primary and metastatic prostate cancer is the crucial first step in selecting appropriate targets for immune attack. In this study, the distribution of the tumor-associated antigens GM2, Tn, sTn, Thompson-Friedenreich antigen (TF), Globo H, Le^y, MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC7, carcinoembryonic antigen, β chain of human chorionic gonadotropin (hCGβ), HER2/neu, PSMA, and KSA on primary and metastatic prostate cancer and 16 types of normal tissues was compared by immunohistochemistry, using a panel of well-characterized monoclonal antibodies. Our results show that GM2, KSA, and MUC2 were strongly expressed on 8 or 9 of 9 metastatic prostate cancer biopsy specimens and, with PSMA, hCGβ, TF, Tn, and sTn, on 8 or more of 11 primary prostate cancer specimens. Tn, MUC1, and PSMA were expressed on 4–6 of 9 metastatic specimens. The remaining antigens were expressed on no more than three of nine metastatic specimens. Normal tissues were also tested with all antibodies. With regard to the eight antigens most widely expressed on prostate cancers, PSMA was not expressed significantly on any of the normal tissues except prostate epithelium. Tn, sTn, hCGβ, and MUC2 were detected on up to 3 of 10 types of normal epithelia. GM2, TF, MUC1, and KSA were more broadly distributed on normal epithelia, all primarily at the secretory borders. STn, KSA, and hCGβ were also detected in the testis, and GM2 was expressed on gray matter of brain. From the 30 antigens that we have screened, this study provides the basis for selecting GM2, TF, Tn, sTn, hCGβ, MUC1, MUC2, KSA, and PSMA as target antigens for specific immunotherapy of prostate cancer.

INTRODUCTION

The progression of prostate cancer from the hormone-naïve primary to increasingly androgen-independent metastatic lesions is associated with a number of molecular and genetic changes. These changes can affect the expression of specific antigens on the cell surface. Defining the expression of tumor-associated antigens on prostate cancers of different stages is the crucial first step in selecting targets for specific immunotherapy. We have previously used a panel of murine mAbs[3] to determine the expression of 18 different tumor-associated carbohydrate antigens on a broad range of malignancies, including five hormone-naïve primary prostate cancer specimens, by immunohistochemistry (1, 2). GM2, Tn, sTn, and TF were expressed in four or five of five primary prostate cancers, Le^y was expressed on three of five and Globo H was expressed on two of five primary prostate cancers. Twelve other antigens were expressed on one or none of the five specimens. Metastatic prostate cancer specimens were not tested.

In this report, we have (*a*) increased the number of primary hormone-naive cancers evaluated; (*b*) extended the work to include metastatic lesions; (*c*) evaluated the expression on this expanded tumor panel of the six carbohydrate antigens expressed on more than one of five primary cancers; and (*d*) evaluated the expression of 12 protein tumor antigens: MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B, MUC7, hCGβ, HER2/neu, PSMA, KSA, and CEA. This study provides a comprehensive basis for selecting cell surface target antigens for specific immunotherapy of prostate cancers with mAbs or vaccines.

MATERIALS AND METHODS

Tissue Samples. Frozen specimens embedded in Tissue-Tek OCT compound (Diagnostic Division, Elkhart, IN) were provided with pathological reports by the Tissue Procurement Service of MSKCC, with the exception of four frozen specimens of metastatic prostate cancer kindly provided by Dr. G. Steven Bova (Pelican Laboratory, Johns Hopkins University). Cryostat sections were cut at 5 μm, dried in air, and fixed with neutral buffered 10% formalin solution (Sigma Chemical Co., St. Louis, MO) for 10 min before H&E or immune staining.

mAb and Immunohistochemistry. The murine mAbs and the antigens they recognize are summarized in Table 1. mAb 696 was provided by Nobuo Hanai (Kyowa Hakko Kogyo Co., Tokyo, Japan); 1E3 by A. K. Singhal (The Biomembrane Institute, Seattle, WA); B72.3 and CC49 by J. Schlom (National

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[1] This work was supported by CaPCURE, the Cancer Research Institute, The PepsiCo Foundation, and NIH Grants RO1 CA 61422, POI CA 33049, and CA 05826.

[2] To whom requests for reprints should be addressed, at Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Fax: (212) 794-4352.

[3] The abbreviations used are: mAb, monoclonal antibody; ABC, avidin-biotin complex; CEA, carcinoembryonic antigen; PSMA, prostate-specific membrane antigen; TF, Thompson-Friedenreich antigen; hCGβ, β chain of human chorionic gonadotropin; MSKCC, Memorial Sloan-Kettering Cancer Center.

Table 1 Mouse mAbs studied

mAb	Immunoglobulin Class	Antigen	Antigen structure	Ref.
696	IgM	GM2	GalNAcβ1→4(NeuAcα2→3) Galβ1→4Glcβ1→1Cer	3
49H.8	IgM	TF	Galβ1→3GalNAcα-*O*-serine/threonine	4
1E3	IgG2b	Tn	GalNAcα-*O*-serine/threonine	Unpublished[a]
B72.3	IgG1	sTn	NeuAcα2→6GalNAcα-*O*-serine/threonine	5
MBr1	IgM	Globo H	Fucα1→2Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer	6
3S193	IgG3	Le^y	Fucα1→2Galβ1→4(Fucα1→3)GlcNAcβ1→3Gal	7
HMFG-2	IgG1	MUC1	VTSAPDTRPAPGSTAPPAHG repeating	8
LDQ10	IgM	MUC2	PTTTPISTTTTVTPTPTPTGTQT repeating	9
M3.2	IgG2a	MUC3	HSTPSFTSSITTTETTS repeating	10
MUC4.275	IgG	MUC4	TSSASTGHATPLPVTD repeating	10, 11
CLH2	IgG1	MUC5AC	TTSTTSAP repeating (interrupted)	12
PANH2	IgG1	MUC5B	No peptide repeats	13, 14
PANH3	IgG1	MUC7	TTAAPPTPSATTPAPPSSSAPPE repeating	13, 14
NCL-CEA	IgG1	CEA	Glycoprotein (M_r 180,000)	Vector Co.
Cyt351	IgG	PSMA	Protein (M_r 100,000)	15–17
GA733-2	IgG2a	KSA(EGP-2)	Glycoprotein (M_r 40,000)	18
FB12	IgG1	hCGβ	145-amino acid glycoprotein	19
NCL-CBE1	IgG2a	HER2/neu	Protein (M_r 185,000)	20

[a] A. Singhal and S. Hakomori, unpublished data.

Cancer Institute, Bethesda, MD); 49H.8 by R. Koganty (Biomira Inc., Edmonton, Alberta, Canada); MBr1 by M. I. Colnaghi (Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy); 3S193 by L. J. Old (MSKCC); HMFG-2 by J. Taylor-Papadimitriou (Imperial Cancer Research Fund, London, United Kingdom); LDQ10 by F. X. Real (Institut Municipal d'Investigacio Medica, Barcelona, Spain); M3.2 and MUC4.275 by V. Apostolopoulos (Austin Research Insitute, Heidelberg, Victoria, Australia); CLH2, PANH2, and PANH3 by H. Clausen (University of Copenhagen, Copenhagen, Denmark); Cyt351 by W. Heston (MSKCC); FB12 by D. Bellet (Institut Gustave-Roussy, Villejuif, France); and GA733–2 by D. Herlyn (The Wistar Institute, Philadelphia, PA). mAbs NCL-CEA and NCL-CBE1 were purchased from Vector Laboratories, Inc. (Burlingame, CA).

The ABC immunoperoxidase method was performed as described previously (21). Briefly, the sections were quenched with 0.1% H_2O_2 in PBS for 15 min, blocked with avidin and biotin reagents (Vector Laboratories, Inc.) for 10 min each, incubated in 10% serum of horse or goat from which the second antibody was raised, and incubated with various mAbs for 1 h at optimal concentration. The optimal mAb concentration was selected based on strong reactivity against the known positive target cells and little or no background against stroma. The concentrations of mAbs used were as follows: FB12 at 0.5 μg/ml; 696 at 0.8 μg/ml; B72.3, CC49, Cyt351, and GA733–2 at 2 μg/ml; 49H.8 at 5 μg/ml; MBr1 at 1 μg/ml; 3S193 at 1.5 μg/ml; HMFG-2, M3.2, MUC4.275, CLH2, PANH2, PANH3, and 1E3 (supernatants) at between 1:3 and 1:6; LDQ10 (ascites) and NCL-CBE1 at 1:15; and NCL-CEA at 1:50. The sections were subsequently incubated with 1:600 biotinylated horse antimouse IgG or 1:300 goat antimouse IgM antibodies (Vector Laboratories, Inc.) for 40 min and then incubated in 1:50 ABC reagent (Vector Laboratories, Inc.) for 30 min. Reactions were developed with 0.02% H_2O_2 and 0.1% diaminobenzidine tetrahydrochloride (Sigma) for 2–5 min. Slides were then counterstained with Harris modified hematoxylin (Fisher Scientific, Fair Lawn, NJ) for 1–3 min. The immunoreactivities were graded based on the percentage of positive cells and staining intensity above that seen on the negative control: 1+ (weak), 2+ (moderate), 3+ (strong), and 4+ (very strong). Staining intensities of 2+ or stronger were considered positive (see Table 2 and Fig. 1). Known positive and negative control slides were used in each experiment. Results with the several IgM, IgG3, and IgG2 mAbs included in the panel of antibodies tested ruled out nonspecific adherence of particular subclasses of antibodies.

An indirect immunoperoxidase assay was performed on normal liver, kidney, and stomach samples. These tissues reacted strongly with ABC reagent directly, producing high background staining. Briefly, the sections were quenched with 0.1% H_2O_2 in PBS for 15 min, blocked with 10% serum, and incubated with mAbs for 1 h at the optimal concentration. The sections were incubated with 1:100 rabbit antimouse immunoglobulin labeled with peroxidase (DAKO Co., Carpinteria, CA) for 1 h and developed as described for the ABC method.

RESULTS

Expression of Tumor-associated Antigens on Prostate Cancer. Using 50% or more of tumor cells positive per tissue section as a cutoff, GM2, KSA, MUC1, MUC2, and PSMA were expressed on five or more of nine metastatic prostate cancer specimens, and using 20% of positive cells as cutoff, GM2, KSA, MUC2, and Tn were expressed in eight or more of nine specimens (see examples of staining in Fig. 1 and the results summarized in Table 2). TF, sTn, Globo H, hCGβ, and Le^y were expressed (using the 50% cutoff) on two or three of nine metastatic specimens, and CEA, HER2/neu, MUC3, MUC4, MUC5AC, MUC5B, and MUC7 were all expressed in one or none of the specimens. Primary prostate cancers expressed GM2, KSA, TF, Tn, sTn, MUC2, hCGβ, and PSMA in 8 of 11 or more specimens, whereas CEA, Le^y and Globo H were expressed in 2–7 of 11. MUC3, MUC4, MUC5AC,

Table 2 Proportion of normal and tumor specimens with 50% (20%) or more of cells positive by immunohistology[a]

Tissue	Antigen (mAb)																			
	GM2 (696)	TF (49H.8)	Tn (1E3)	sTn (B72.3)	sTn (CC49)	Globo-H (MBr1)	Le^y (3S193)	MUC1 (HMFG-2)	MUC2 (LDQ10)	MUC3 (M3.2)	MUC4 (M4.275)	MUC5AC (CLH2)	MUC5B (PANH2)	MUC7 (PANH3)	CEA (NCL-CEA)	PSMA (Cyt351)	KSA (GA733-2)	bCGβ (FB12)	HER2/ (NCL-CBE1)
Metastatic prostate cancer	8/9	2/9	4/9	3/9	3/9	2/9	3/9	5/9	8/9	0/9	0/9	1/9	0/9	0/9	0/9	6/9	9/9	1/5	1/5
	(8/9)	(5/9)	(8/9)	(4/9)	(4/9)	(2/9)	(3/9)	(5/9)	(9/9)	(1/9)	(0/9)	(1/9)	(1/9)	(0/9)	(2/9)	(6/9)		(2/5)	(1/5)
Primary prostate cancer	11/11	10/11	10/11	6/11	9/11	2/11	4/11	0/11	8/11	0/11	0/11	1/11	0/11	0/11	7/11	8/11	11/11	10/11	0/11
		(10/11)	(10/11)	(9/11)	(10/11)	(6/11)	(6/11)	(3/11)	(8/11)	(0/11)	(0/11)	(1/11)	(1/11)	(0/11)	(9/11)	(10/11)		(10/11)	(0/11)
Prostate glandular epithelia	6/6	0/2	0/6	0/6	0/6	3/6	2/6	1/6	2/6	0/6	0/6	1/6	0/6	0/6	4/6	3/6	6/6	3/6	0/6
			(2/6)	(2/6)	(1/6)	(4/6)	(3/6)	(2/6)	(5/6)	(2/6)	(1/6)	(1/6)	(0/6)	(0/6)	(5/6)	(5/6)		(3/6)	(0/6)

[a] All of the tumor tissues were stained by ABC immunoperoxidase methods.

MUC5B, MUC7, and HER2/neu were detected on one or none of the specimens tested. Interestingly, MUC1 was present on five of nine metastatic prostate cancers but was not strongly expressed on any primary cancers.

The correlation between staining intensity and percentage of positive tumor cells in the nine metastatic specimens was determined (see Table 4). GM2, MUC2, and KSA stained 60–95% of tumor cells in at least eight of nine metastatic prostate cancer specimens, generally with strong or very strong staining. These antigens were also expressed on at least 90% of most primary prostate cancers, with a staining intensity of 4+ (data not shown). Six of nine metastatic specimens also expressed PSMA with 3+ or greater intensity. Samples of the staining against these antigens on metastatic prostate cancer specimens and the corresponding percentage of positive cells and staining intensity are demonstrated in Fig. 1. The lack of background staining on normal stroma or adjacent normal tissues was a universal finding with these antibodies used at these concentrations.

Expression of Tumor-associated Antigens on Normal Tissues. In addition to normal prostate tissue, many other normal tissues were tested. Examples are shown in Fig. 2 and the results are summarized in Table 3. GM2 was distributed on gray matter of brain and the epithelia of all tested organs except liver. Tn was expressed on epithelia of stomach and ovary. sTn, defined by mAb B72.3 and CC49, was expressed on Leydig cells of testis and on ovarian and gastric epithelia. mAb CC49 also reacted with epithelia of colon and pancreas. TF was detected on 5 and Globo H and Le^y were detected on 7 epithelia of the 10 tested. MUC1 was weakly distributed on the epithelia of all of the tested organs except liver. MUC2 was observed on the epithelia of prostate, colon, and pancreas. MUC3 was only detected on epithelia of pancreas. MUC4 was expressed on epithelia of colon and prostate (weakly). MUC5AC was strongly expressed in stomach epithelium. MUC7 and HER2/neu were not expressed on any normal tissues, and MUC5B was only detected on normal colon epithelium and, weakly, in the testis. hCGβ was detected in epithelia of prostate, stomach, and pancreas, and weakly in colon and lung, and it was detected in the testis. PSMA was only detected on prostate epithelia and, weakly, on skeletal muscle cells. KSA was strongly expressed on the epithelia of all of the tested organs except stomach and liver, and it was moderately expressed on seminiferous tubules of testis. The pattern of expression of each of these antigens on normal epithelia was mainly luminal, with polarity evident. Antigen expression was primarily at the luminal surface of positive cells.

DISCUSSION

Immunohistology is notoriously inconsistent for quantitating antigen expression, even when performed by an experienced investigator, because the results obtained are dependent on precise mAb specificity, affinity, and concentration. We have attempted to limit these variables by selecting well-studied mAbs and a consistent method for selecting the optimal concentration of each mAb. In addition, we have not been able to determine by immunohistology whether the antigens are being expressed at the cell surface or intracellularly. Although all of

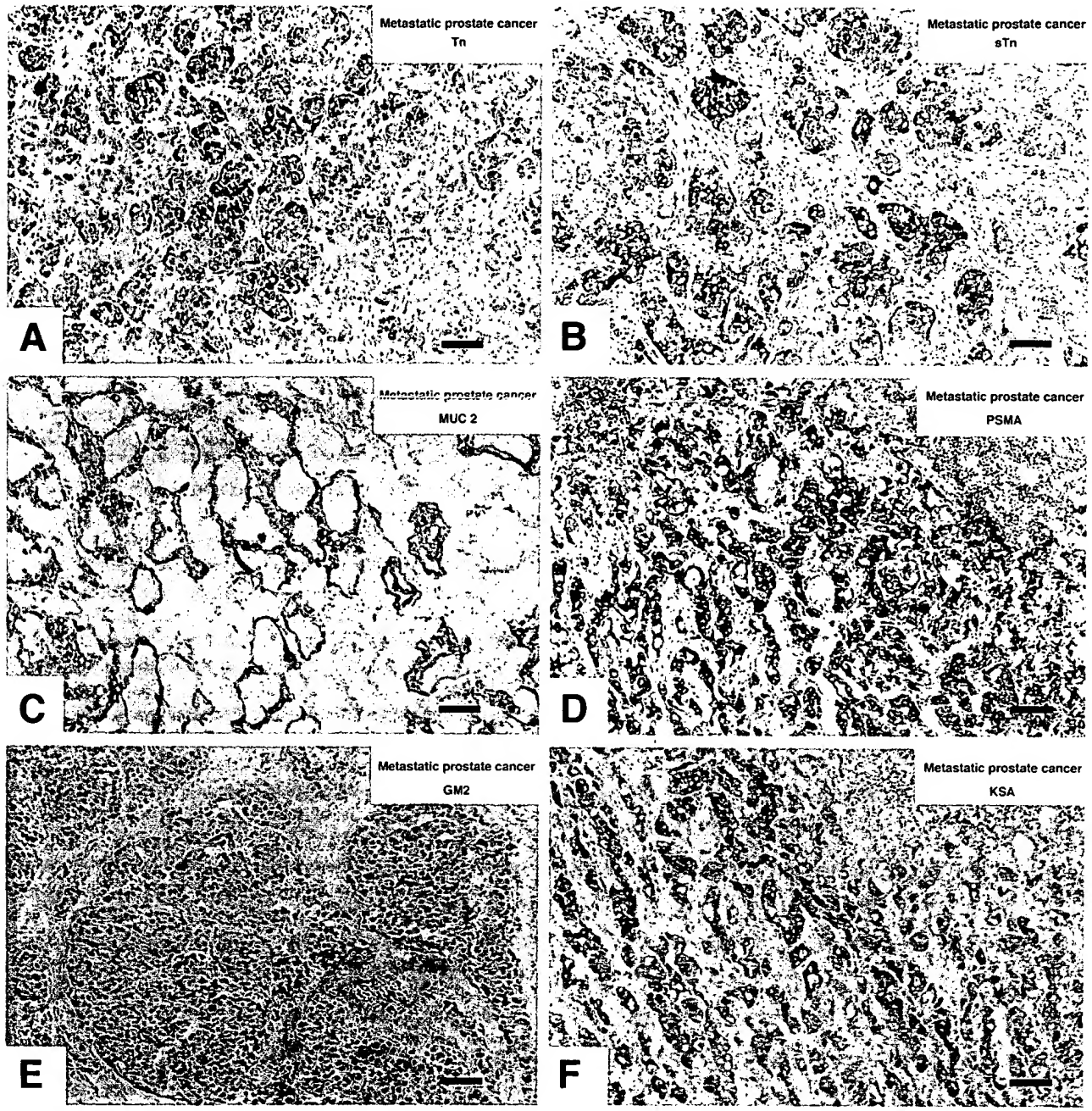

Fig. 1 Expression of tumor-associated antigens on metastatic prostate cancer. Strong immune staining was detected on metastatic prostate cancer in bone marrow with mAb 1E3 against Tn (*A*; 80% 3–4+) and mAb B72.3 against sTn (*B*; 80% 3–4+); in lung with mAb LDQ10 against MUC2 (*C*; 80% 4+); and in lymph node with mAb Cyt351 against PSMA (*D*; 95% 4+), mAb 696 against GM2 (*E*; 90% 4+), and mAb GA733–2 against KSA (*F*; 95% 3+) the absence of staining on stroma. *Scale bar*, 100 μg.

the antigens selected (with the exception of hCGβ and mucins MUC2–7) are known to be generally expressed at the cell surface, our studies cannot confirm this in these particular cancers and normal tissues. With these provisos, this study identified nine prostate cancer cell surface antigens that are candidate targets for immune attack. The criteria for selecting target antigens for study in immunotherapy trials depends on the frequency of staining in specimens from different patients' primary and metastatic tumors, as well as the percentage of cells positive in each specimen and the staining intensity. It also includes the specificity of this staining, including the distribution pattern of the antigen on various normal tissues. GM2, KSA, and MUC2 were expressed strongly on at least 8 of 9 metastatic prostate cancer specimens and 8 of 11 primary cancers. Tn, MUC1, and PSMA were strongly expressed in between 4–6 of the 9 metastatic specimens, and TF, Tn, sTn, hCGβ, and

Table 3 Antigen expression on normal tissues defined by immunohistology[a]

Normal tissue (No.)[b]	Antigen (mAb)																		
	GM2 (696)	TF (49H.8)	Tn (1E3)	sTn (B72.3)	sTn (CC49)	Globo H (MBr1)	Le^y (3S193)	MUC1 (HMFG-2)	MUC2 (LDQ10)	MUC3 (M3.2)	MUC4 (M4.275)	MUC5AC (CLH2)	MUC5B (PANH2)	MUC7 (PANH3)	CEA (NCL-CEA)	PSMA (Cyt351)	KSA (GA733-2)	hCGβ (FB12)	HER/ NCL-CBE1
Brain (3)																			
Gray matter	2+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
White matter	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen (2)																			
White pulp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Red pulp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2+[c]	–	–	–	–
Lymph node (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Striated muscle (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+/–	–	1+	–
Smooth muscle (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–
Epithelia																			
Lung (2)	3+	2+	–	–	–	3+	2+	2+	–	–	–	–	–	–	1+	–	4+	1+	1+
Breast (2)	3+	–	–	–	–	3+	2+	1+	–	–	–	–	–	–	2+	–	3+	–	–
Prostate (6)	4+	2+	+/–	+/–	+/–	2+	3+	+/–	2+	–	+/–	–	–	–	3+	3+	4+	2+	2+
Colon (2)	4+	2+	–	–	2+	–	–	2+	3+	–	3+	–	3+	–	3+	–	4+	1+	–
Stomach (2)	3+	2+	2+[d]	2+(½)	2+(½)	3+	3+(½)	1+	–	–	–	4+	–	–	–	–	–	3+	–
Pancreas (2)	3+	–	–	–	2+(½)	4+	3+	2+	2+	2+	–	–	–	1+	–	–	4+	2+	–
Uterus (2)	3+	–	–	–	–	4+	2+	1+	–	–	–	–	–	–	1+	–	4+	–	–
Ovary (2)	3+	2+	2+(½)[e]	3+(½)	3+(½)	4+	3+(½)	1+	–	–	–	–	–	–	–	–	2+	–	–
Liver (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Kidney (2)	2+	–	–	–	–	–	–	2+	–	–	–	–	–	–	–	–	1+	–	–
Testis (2)			–	3+[f]	3+[f]		–	–	–	–	–	–	1+[g]	–	–	–	3+[g]	2+	–
Connective tissues																			
Lung (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Breast (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Prostate (2)	1+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Colon (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Stomach (2)		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pancreas (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Uterus (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ovary (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Kidney (2)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

[a] All tissues were stained by the ABC immunoperoxidase method except stomach, liver, and kidney, which were stained by the indirect immunoperoxidase method.
[b] The number in parentheses indicates the number of different specimens tested.
[c] Histocytes in the red pulp were predominantly stained.
[d] A few luminal cells were stained.
[e] (½), one of the two specimens was positive.
[f] Leydig cells were stained.
[g] Seminiferous tubules were stained.

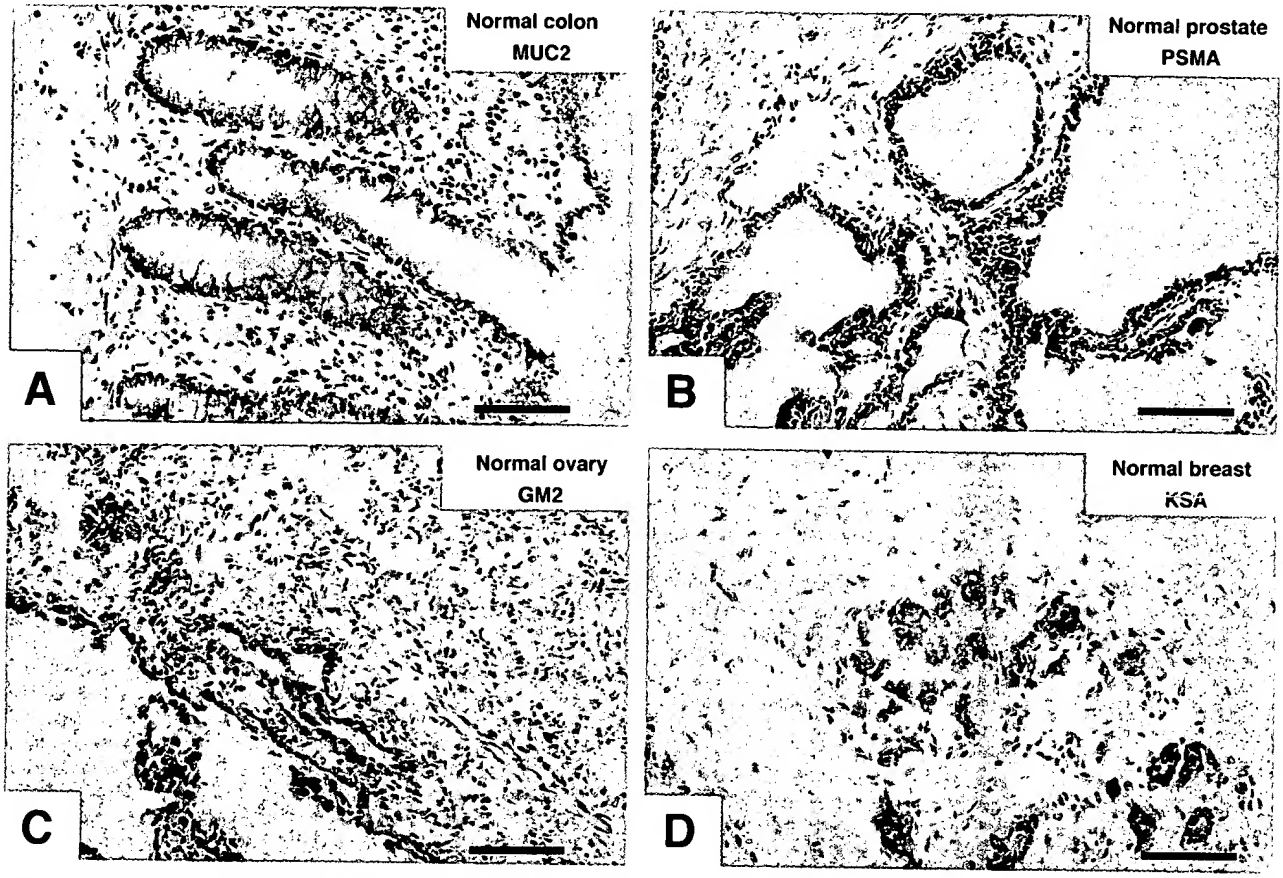

Fig. 2 Expression of the tumor-associated antigens on normal tissues. Luminal cells of normal epithelia were stained in colon with mAb LDQ10 against MUC2 (*A*), in prostate with mAb Cyt351 against PSMA (*B*), in ovary with mAb 696 against GM2 (*C*), and in breast with mAb GA733–2 against KSA (*D*). Note the heterogeneous expression of PSMA on prostate epithelia. *Scale bar,* 100 μg.

PSMA were strongly expressed in 8–10 of 11 primary cancers. The frequently strikingly positive imaging of prostate cancers with sTn mAbs B72.3 and CC49 (22) and the immunohistological results in a recently completed study with anti-PSMA mAb CYT351 demonstrating strongly positive staining in seven of eight lymph node metastases (16, 17) have suggested that these two antigens are widely expressed on prostate cancers. Although there are no previous studies demonstrating the expression of GM2, KSA, TF, Tn, or MUC2 on prostate cancers, the surprising frequency and intensity of their staining on most of the primary and metastatic prostate carcinomas that we tested leave little doubt about their presence in prostate cancers and their suitability as potential targets for immune attack.

The widespread expression of most of these antigens on normal tissues is, at first look, disturbing. GM2 and KSA are expressed on the epithelial surfaces of nearly all tissues tested, whereas PSMA, hCGβ, TF, Tn, sTn, and MUC2 showed weak to moderate staining on epithelial cells of between one and five organs. The only other expression of these antigens on normal tissues was moderate expression of GM2 in brain gray matter and expression of sTn, HCGβ, and KSA in the testis. There are now sufficient data from clinical trials with vaccine-induced antibody responses against GM2, GD2, MUC1, HCGβ, Globo H, TF, and sTn antigens (23–30) and passive administration of mAbs against GD2, GD3, KSA, Globo H, and sTn (31–35) to draw conclusions about the consequences of antigen distribution on normal tissues. GM2, GD2, and GD3 exposure on cells in the brain (both GD2 and GD3 are more abundantly expressed than GM2), and GM2, sTn, MUC1, HCGβ, and Globo H antigen expression in cells at secretory borders of epithelial tissues induced neither immunological tolerance nor autoimmunity once antibodies were present, suggesting that they are sequestered from the immune system. Our experience in patients with prostate cancer supports this suggestion. No normal organ toxicity was seen in prostate cancer patients producing antibodies against MUC1 or Globo H after immunization with conjugate vaccines,[4] nor were normal epithelial cells detectably affected by treatment with ^{131}I-labeled mAb against sTn (22). Treatment with mAbs against GD2 and GD3 has not induced central nervous system toxicity in children or adults (31–33). On the other hand, treatment with mAbs against some antigens present in locations other than brain and secretory epithelia have resulted in toxicity: high doses of some mAbs against GD2 (which is also expressed in some peripheral nerves) has resulted in

[4] S. Slovin, H. Scher, and P. Livingston, unpublished observations.

Table 4 Correlation between expression of tumor-associated antigens on nine metastatic prostate cancers

Metastatic site	GM2 696	TF 49H.8	Tn 1E3	sTn B72.3	MUC1 HMFG-2	MUC2 LDQ10	hCGβ FB12	PSMA Cyt351	KSA GA733-2
	95%	90%	85%	90%	60%	80%	–	–	60%
Bone	3+	3+	3–4+	3–4+	2–4+	2+			2–3+
	90%	40%	80%	80%	5%	80%	–	80%	95%
Lung	4+	1+	2–3+	3–4+	3+	4+		3–4+	4+
	90%	70%	70%	30%	90%	90%	80%	–	95%
Lymph node	4+	4+	2–3+	2–3+	3–4+	4+	4+		4+
	60%	–	20%	–	–	40%	30%	90%	95%
Lymph node	2–3+		2+			2+	1+	4+	3–4+
	60%	40%	20%	5%	10%	90%	–	95%	95%
Lymph node	2–4+	1+	2+	2+	2+	3+		4+	4+
		40%	20%	–	80%	95%		95%	90%
Lymph node	–	2+	2+		3+	4+		4+	3+
	90%	40%	–		70%	90%		95%	95%
Liver	2+	2+			3+	4+		4+	4+
	95%	30%	20%	90%	70%	90%		5%	95%
Liver	3+	2+	2+	4+	4+	4+		4+	4+
	90%	–	50%	–	–	90%		90%	95%
Brain	3+		2+			3+		4+	4+

peripheral neuropathies (36), and treatment with a mAb against Le^x (expressed at secretory borders but also on polymorphonuclear leukocytes) has resulted in striking, short-lived neutropenia (37, 38). Against this background, nine antigens (GM2, KSA, TF, Tn, sTn, hCGβ, PSMA, MUC1, and MUC2) all appear to be excellent targets for immunotherapy of prostate cancer with vaccines or mAbs.

These results provide the basis for using combinations of antibodies or for polyvalent vaccines for immunotherapy of prostate cancer. We have screened for the expression of 30 different potential prostate cancer cell surface antigens (including 18 here and 12 additional antigens in our previous studies; Refs. 1 and 2) to identify the 9 most widely expressed antigens. However, none of the nine were strongly expressed on every prostate cancer cell, suggesting the need for a polyvalent vaccine or mixture of mAbs for immunotherapy. For instance, the four cases of metastatic prostate cancer with weak or moderate GM2 expression in our study showed strong expression of PSMA and KSA (see Table 4). All metastatic lesions showed strong expression of at least two of these eight antigens in at least 80% of tumor cells and strong expression of at least one additional antigen in at least 60% of cells. On the basis of these results, we have initiated a program aimed at augmenting the immune response against each of these nine antigens with a polyvalent prostate cancer vaccine.

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RECOMBINANT ONCOTOXIN AR209 (ANTI-P185^{erbB-2}) DIMINISHES HUMAN PROSTATE CARCINOMA XENOGRAFTS

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ABSTRACT

Purpose: Prostate cancer is the most common malignancy of males in the United States. Although the overall survival rate for early stage prostate cancer is good, if cancer recurs following curative therapies there is no adequate salvage therapy. Systemic chemotherapy has never been associated with any meaningful improvement in overall survival or overall objective benefit. There is a need to develop novel therapies for prostate cancer.

Materials and Methods: Two prostatic cancer cell lines, DU-145 and PC-3, were grown as subcutaneous xenografts in athymic nude mice. The recombinant oncotoxin AR209, formerly OLX-209 [e23(Fv)PE38KDEL]), has the specificity of an anti-p185^{erbB-2} antibody contained within a single-chain antibody domain (e23Fv) coupled to a portion of the *Pseudomonas* exotoxin A (PE38KDEL). Using Western blot analysis, the cell lines were shown to express p185^{erbB-2}. The mice received either 3 i.v. injections, one every 2 days, of the recombinant oncotoxin AR209 or PBS, or were implanted with osmotic pumps that delivered a constant s.c. amount of AR209 or PBS.

Results: The oncotoxin was effective in reducing the size of s.c. prostatic xenografts in athymic nude mice. The data demonstrated that small tumors (<200 mm.^{3}) were effectively reduced in size. However, larger tumors (>500 mm.^{3}) were not effectively diminished.

Conclusions: This study provides preliminary evidence for the utility of a recombinant oncotoxin in the treatment of prostate carcinoma. Recombinant oncotoxins may be an effective clinical addition for the management of metastatic prostate lesions in patients treated with conventional therapy.

KEY WORDS: HER-2, *neu*, *Pseudomonas* exotoxin A, recombinant toxin, adenocarcinoma

Prostate cancer has the highest incidence rate of all malignancies for males in the United States.[1] A predicted 334,500 new cases of prostate cancer were diagnosed in 1997 resulting in 41,800 deaths.[1] The incidence rate for prostate cancer increased by 50% from 1983 to 1995[2] with a concomitant 40% increase in mortality.[3] The overall survival rate for early stage prostate cancer is good. However, if cancer recurs following curative therapies, there is no adequate salvage therapy.[4,5] For localized disease, current treatments for prostate cancer include radical prostatectomy or definitive radiation therapy. Although advances in surgical techniques have reduced morbidity, significant side effects include incontinence, rectal injury, and impotence.[4] More advanced stage D1 tumors are usually treated with the "watch and wait" method, although there are some reports that hormonal therapy may be of benefit to some patients.[4] For metastatic disease, androgen ablation therapy is most often used. This type of therapy dates back 55 years,[6] and "the principles of metastatic prostate cancer therapy have remained basically unchanged since."[4] This therapy often includes bilateral orchiectomy (physical castration), which results in significant psychological side effects. Medical "castration" using diethylstilbestrol, luteinizing hormone-releasing hormone analogs, and anti-androgen therapy (flutamide, cyproterone acetate) is often used as an alternative to bilateral orchiectomy; however, a significant number of prostate cancers become refractory to hormonal therapy, drastically decreasing survival.[4,5] Systemic chemotherapy has never been associated with any significant enhancement in overall survival.[7,8] For patients with hormone-refractory tumors, the clinician's main responsibilities are to manage pain, to provide adequate nutrition, and to preserve mobility. Although there are some experimental therapies for prostate cancer such as the use of suramin[9] or somatostatin analogs,[10] there is an urgent need to design new therapies based on our knowledge of the molecular biology of prostate cancer.

Immunotherapy has proven to be an intriguing possible replacement for traditional cytotoxic therapy. One approach to immunotherapy involves the direction of toxins or radionuclides to a cancer cell by targeting a specific protein on the tumor cell's surface. This "silver bullet" approach to therapy is particularly attractive for several reasons. First, the therapy will have few of the side effects associated with traditional cytotoxic therapy. Second, the therapy will not rely on the increased growth rate of cancer cells versus normal cells; quiescent or slow-growing tumors, like many prostate tumors, prove difficult to treat with traditional therapy.[7,8] Third, micrometastatic lesions that are often undetected by surgeons can be specifically targeted before they become a clinical concern.

Recombinant oncotoxins are the "silver bullet" therapy of modern immunotherapy. The traditional recombinant oncotoxins are made to antigens that are expressed solely by tumor cells and not by normal cells. Alternatively, recombinant oncotoxins can be designed to target proteins that are

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expressed on normal cells providing they are overexpressed on tumor cells as well. One example of this type of recombinant oncotoxin is AR209. This drug targets cells that express p185^{erbB-2}, the protein product of the c-*erb*B-2 (HER-2/*neu*) protooncogene. We[11] and others[12] have previously demonstrated that the AR209 compound is specifically targeted to cells that express the p185^{erbB-2} protein. In the case of AR209, protein synthesis in target cells is inhibited by the addition of a modified *Pseudomonas* exotoxin A.[12] It has been shown that many tumor types express moderately to extremely high levels of p185^{erbB-2}, including non-small cell lung carcinoma,[11,13,14] gastric carcinoma,[15] breast adenocarcinoma,[16] and prostate cancer.[2,17–23] We have previously demonstrated that AR209 effectively diminishes subcutaneous and orthotopic human lung cancer xenografts in nude mice.[11,24] In this study, we evaluated the efficacy of AR209 on human prostate xenografts grown subcutaneously in athymic nude mice.

MATERIALS AND METHODS

Cell lines and culture conditions. The prostate carcinoma cell lines LNCaP[25] and PC-3[26] were propagated in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS, Life Technologies). The prostatic cancer cell line DU-145[27] was grown in Eagle's Minimum Essential Medium (EMEM, Life Technologies) supplemented with 10% FBS. For s.c. injections, cells were isolated by trypsinization (Life Technologies) and centrifugation at 500 *g* for 10 minutes, washed once in medium supplemented with 10% FBS and washed an additional 2 times with phosphate-buffered saline (PBS, Life Technologies). Cells were suspended in PBS for injections at 2×10^6 cells/0.1 ml.

Western blot analysis. Cells were prepared for western blot analysis as previously described.[11] Briefly, cells were grown to 80% confluence, washed 3 times with PBS, and lysed by scraping in 0.5 ml. of sample buffer (0.5 M Tris-Cl, pH 6.8, 1% SDS, 10% glycerol, and 0.01 gm./ml. bromophenol blue), followed by sonication. After denaturation by boiling in 1% β-mercaptoethanol, equal amounts of protein were loaded and separated on an SDS-PAGE gradient gel (4 to 15% Tris-glycine, Bio-Rad, Hercules, CA). Proteins were transferred to nitrocellulose (Bio-Rad). The blots were blocked in 1% milk and 1% bovine serum albumin (BSA). The mouse anti-human p185^{erbB-2} monoclonal antibody OM-11-952A (Genosys Biotechnologies, Inc., The Woodlands, TX) was incubated with blots at 6.25 μg./ml. The blots were detected using Immun-Lite II chemiluminescent protein detection system (Bio-Rad).

Experimental animals. Specific pathogen-free, 4 to 6 weeks old male *nu/nu* (nude) mice obtained from Harlan, Sprague, Dawley (Indianapolis, IN) were housed in sterilized filter-topped cages (Laboratory Products, Inc., Maywood, NJ), kept in laminar flow isolators (Forma Scientific, Marietta, OH) and fed autoclaved food and water ad libitum. Mice were acclimated for 1 week prior to use in study protocols. All procedures involving the animals were performed under sterile conditions in a laminar flow hood (Forma Scientific). All studies were approved by the LSUMC Institutional Animal Care and Use Committee.

In vivo antitumor assay. Tumor cells (2×10^6 per mouse in PBS) were injected s.c. into the flanks of athymic nude mice. In all experiments, tumors were allowed to establish and grow before therapy was initiated. AR209 was administered to mice using 1 of 2 methods. 1) Three i.v. injections of AR209 at 86 μg./kg. or PBS, once every 2 days, were administered. The LD_{10} for AR209 on this schedule is approximately 90 μg./kg./dose; the LD_{50} is approximately 160 μg./kg.[11] Further, we have previously demonstrated that mice receiving 86 μg./kg. of AR209 show no adverse side effects of the drug, and grow robustly.[24] Or, 2) 21 days post-implantation, mice containing tumor were randomized into 2 groups. The experimental group (n = 8) received subcutaneous osmotic pumps (ALZA Scientific Products model 2002, Palo Alto, CA) containing 35 μg. AR209 (drug delivery 100 μl./kg./day for 14 days). This resulted in the administration of 2.5 μg. AR209/mouse/day. On day 35 after tumor implantation, the pumps were removed. The placebo group (n = 8) received osmotic pumps containing PBS on the same day. Tumor volumes were monitored using dial calipers. Tumor growth is reported as an average tumor volume, calculated as $\pi^*(w^*l^*h)/2$, where w is width, l is length, and h is height in mm.

Immunohistopathology. Sections were prepared from formalin-fixed paraffin-embedded tumors. After staining with hematoxylin and eosin, each section was examined by a pathologist who was blinded from the experimental conditions used to treat the mouse from whom the tumor section was obtained. Sections were also examined for expression of p185^{erbB-2} using rabbit polyclonal anti-human p185^{erbB-2} (Biomeda #225M, San Mateo, CA). Immunohistochemistry was performed using the Ventana Medical Systems gen II automated immunohistochemistry system and reagents (Tucson, AZ). Diaminobenzidine (DAB) substrate kits supplied and customized by Ventana for horseradish peroxidase as an enzyme marker were used. Immunohistochemical analysis was performed by counting 200 cells (unless the tumor was too small) then taking the percentage of the cells that stained positive and applying the following scale (table): + = 1 to 25% positive, ++ = 26 to 50% positive, +++ = 51 to 75% positive, and ++++ = 76 to 100% positive. A breast adenocarcinoma containing 75 to 100% positively staining cells was used as a positive control for anti-p185^{erbB-2} staining. For determination of necrosis, the following scale was used (table 1): 0 = 0% necrosis, + = 1 to 25% necrosis, ++ = 26 to 50% necrosis, and +++ > 50% necrosis.

RESULTS

Expression of p185^{erbB-2} by prostatic cancer cell lines. Previous studies have demonstrated that p185^{erbB-2} is expressed by primary prostate cancer cells.[2,17–23] Using Western blot analysis, it was confirmed that the prostatic carcinoma cell lines PC-3, LNCaP, and DU-145 expressed the p185^{erbB-2} protein. As seen in fig. 1, each of the cell lines expressed high to moderate levels of p185^{erbB-2}. For comparison, the breast carcinoma cell line T47D and the bronchioloalveolar carcinoma cell line A549 are included on the Western blot. We have previously demonstrated that A549 contains 1 c-*erb*B-2 gene per haploid genome and expresses p185^{erbB-2} at 8.7-fold over normal bronchial epithelium.[11] LNCaP expressed the highest levels of p185^{erbB-2} of the prostatic cell lines. Not all primary tumors will express the high levels of p185^{erbB-2} found in LNCaP. Therefore, to determine if the recombinant oncotoxin AR209 would be efficacious against prostate tumors that express moderate levels of p185^{erbB-2}, PC-3 and DU-145 were used for in vivo animal studies.

AR209 anti-tumor activity. To examine the effect of AR209 on human prostatic tumors that express moderate levels of p185^{erbB-2}, s.c. tumors were established in immunocompromised athymic nude mice. Mice were injected s.c. in the flanks with either DU-145 (n = 14) or PC-3 (n = 16) and the tumors were allowed to establish and grow for 13 days. After 13 days, the mice were randomized into two groups so that the mean tumor volume for each group was comparable. The mean tumor volume for the 2 groups injected with DU-145 was 44.6 mm.^{3} and for the 2 groups injected with PC-3 it was 33.8 mm.^{3} The recombinant oncotoxin AR209 was administered via i.v. injections to half of the mice on days 13, 15, and 17 at 86 μg./kg. As seen in fig. 2, *A*, administration of the drug had a significant impact of the size of DU-145 tumors in the treated mice; however, mice that received i.v. injections of PBS had tumors that were dramatically larger. The same

Immunohistochemical detection of p185^{erbB-2} and histological detetion of necrosis in tumors treated with either AR209 or PBS

Cell Line	Treatment	p185^{erbB-2}	Necrosis
DU-145	Pumps with PBS		
1		++++[a]	++[b]
2		++++	++
3		++++	++
4		++++	++
5		++++	++
6		++	++
7		++++	++
8		+++	+++
DU-145	Pumps with AR209		
1		++++	++
2		+++	++
3		+++	+++
4		+++	++
5		+++	++
6		++	++
7		++++	++
8		+	+
DU-145	3 i.v. injections of AR209		
1		++	+
2		++++	+
3		++	++
4		++++	++
5		++++	+
6		+++	++
PC-3	Pumps with PBS		
1		++	++
2		+++	+
3		+++	+
4		+	+
5		+	+
6		++	+
7		++++	++
PC-3	Pumps with AR209		
1		+	+
2		+	+
3		+	+
4		+	+
5		+	+
6		+	0
7		+	0
PC-3	3 i.v. injections of AR209		
1		++	+
2		++	+
3		+	+
4		+	0
5		+	+
6		++	+
7		++	+

[a] For immunohistochemistry, + = 0–25% positive, ++ = 26–50% positive, +++ = 51–75% positive, ++++ = 76–100% positive.

[b] For necrosis, 0 = 0% necrosis, + = 1–25% necrosis, ++ = 26–50% necrosis, +++ > 50% necrosis.

effect was observed in mice that contained tumors consisting of PC-3 cells (fig. 2, *B*). These data are similar to what was observed in mice containing s.c. lung adenocarcinoma tumors.[11]

Because Phase I/II clinical trials using immunotoxins often use either a bolus[28] or continuous i.v. infusion[29–31] for delivery of drug, we determined the efficacy of AR209 using a continuous s.c. delivery of drug over a 14 day period. Mice were injected s.c. with either DU-145 or PC-3 and tumors were allowed to establish and grow for 21 days. The mice were randomized into two groups for each tumor. The mean tumor volume for mice containing DU-145 tumors was 528.3 mm.^{3} (n = 16) and for mice containing PC-3 tumors it was 142.2 mm.^{3} (n = 16). Osmotic pumps containing AR209 or PBS were implanted as described in Materials and Methods on day 21, and were removed on day 35. As seen in fig. 2, *C*, the drug did not significantly reduce the size of the DU-145 tumors. This is likely due to the large size of the tumors at the initiation of therapy. In support of this hypothesis is the reduction of tumor size seen for PC-3 tumors shown in fig. 2, *D*. These tumors were 3.7-fold smaller than DU-145 tumors at the start of therapy.

Analysis of tumor sections for expression of p185^{erbB-2} and for necrosis. Following treatment with either AR209 or PBS, all mice were sacrificed on day 40 and tumors were resected. The tumors were weighed to confirm the accuracy of volume measurements (data not shown). Sections were made from each tumor, and were analyzed for expression of p185^{erbB-2} and for necrosis (fig. 3). The findings of these studies are shown in the table. The DU-145 cell line homogeneously expressed p185^{erbB-2} as observed by the consistent presence of >75% of the cells expressing p185^{erbB-2} in PBS-treated mice (fig. 3, *A*). A possible concern of using a recombinant oncotoxin is that the drug would preferentially kill only those cells that express the target protein. The continued presence of p185^{erbB-2}-positive cells in AR209-treated mice indicates that the drug is not preferentially killing those cells resulting in a residual tumor containing p185^{erbB-2}-negative cells (fig. 3, *B*). Necrosis was observed in larger tumors (table, pumps delivering PBS or AR209) that was not observed in those treated using i.v. injections of drug. This necrosis is likely a result of the larger tumors outgrowing their blood supply. For PC-3, it appears that p185^{erbB-2} is not homogeneously expressed by all cells (fig. 3, *C*). In 2 sections obtained from different tumors, <25% of the tumor cells in the section expressed p185^{erbB-2}. In mice treated with AR209 delivered by osmotic pumps and containing PC-3 cells, it does appear that the AR209 is preferentially killing only those cells that express the p185^{erbB-2} protein (fig. 3, *D*). In every tumor excised from these mice, <25% of the cells expressed p185^{erbB-2}.

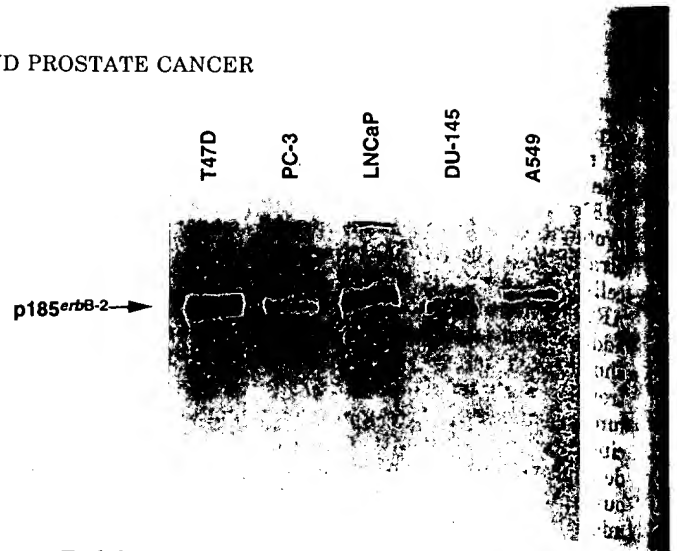

FIG. 1. Each human prostatic carcinoma cell line (PC-3, LNCaP, and DU-145) expresses p185^{erbB-2} protein as demonstrated by Western blot analysis. Protein extracted from breast carcinoma cell line (T47D) and a bronchioloalveolar carcinoma cell line (A549) are also shown for comparison.

DISCUSSION

Because the number of men over the age of 50 is increasing in the United States, the number of cases of prostate cancer has risen significantly in recent years.[4] Between 1989 and 1993, the incidence of prostate cancer increased by 50%.[1] Patients with localized disease (stages A, B, and C) are usually treated with either definitive radiation therapy or with radical prostatectomy, both of which are considered curative. The complications of radiation therapy include diarrhea, proctitis, cystitis, hematuria, rectal bleeding, rectal anal stricture, urethral stricture, rectal ulcer, bowel obstruction, and impotence. The complications of radical prostatectomy include incontinence, urethral stricture, fistula, rectal injury, impotence, and the risk associated with surgical anesthesia. Patients with more advanced disease (stage D1) often progress to systemic disease and are treated with hormonal ablation therapy, which includes bilateral orchiectomy or treatment with diethylstilbestrol, luteinizing hormone re-

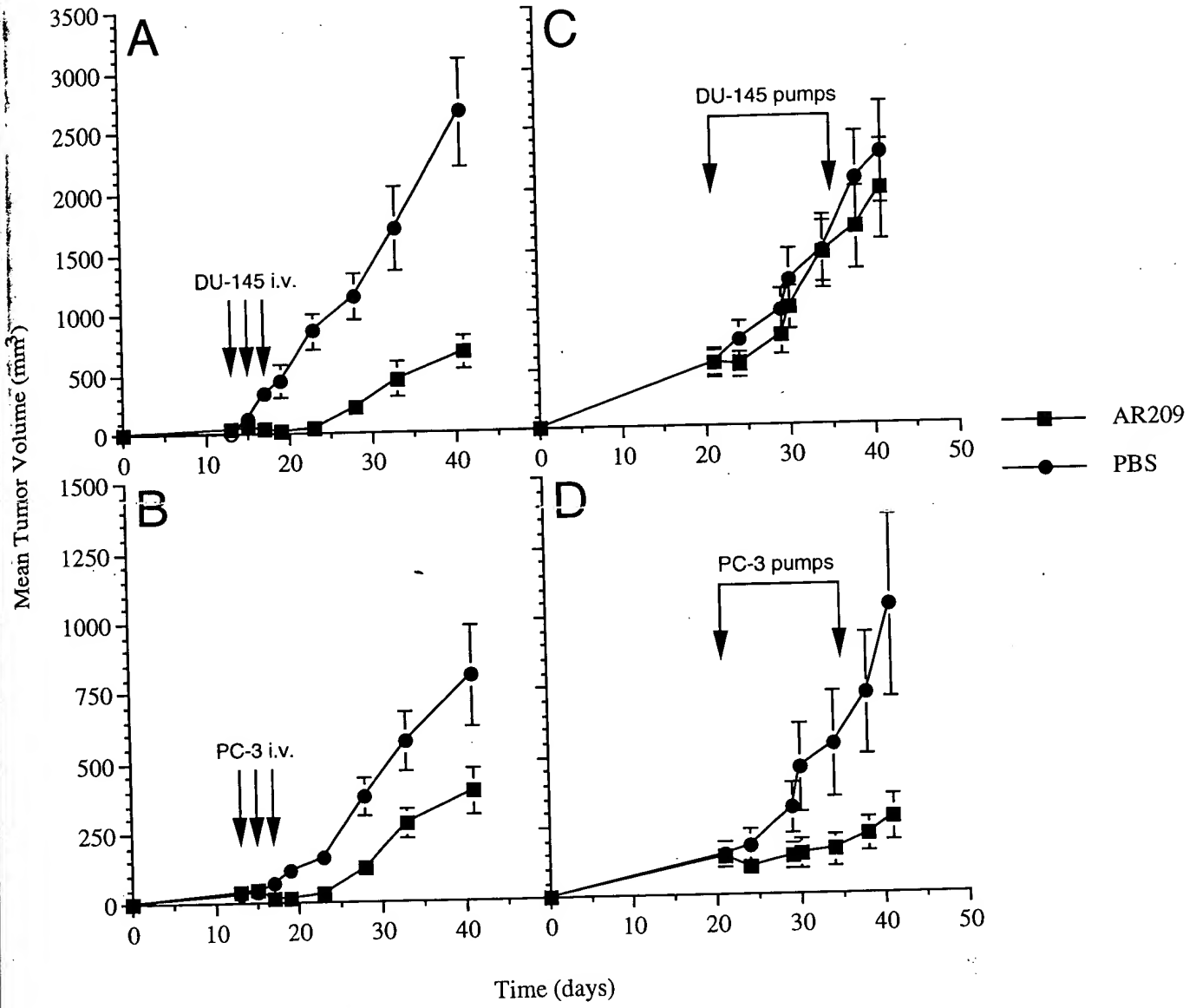

FIG. 2. Treatment of mice containing human prostatic xenografts reduces size of tumors. Mean tumor volume (π*(w*l*h)/2) of mice treated with 3 i.v. injections of AR209 or PBS (*A, C*) or with continuous infusion of AR209 or PBS for 14 days (*B, D*) are shown. Mice contained xenografts of DU-145 (*A, B*) or PC-3 (*C, D*). Error bars represent ± S.E.M.

leasing hormone, or other antiandrogenic agents. The complications of hormonal ablation therapy include hot flashes, psychological effects, cardiovascular risk, gynecomastia, diarrhea, and abnormal liver function. Many patients advance to stage D3, hormone-refractory disease. There is no adequate salvage therapy for these patients, as the use of systemic chemotherapy has never been associated with overall unbiased improvement.[7,8] There are a paucity of data to suggest that the survival of patients with metastatic disease will dramatically improve in the near future. Because of the significant side-effects associated with conventional therapies and the ineffectiveness of salvage therapies, new treatments for prostate cancer must be developed based on our knowledge of the molecular biology of the disease.

It has been established that primary prostatic cancers often overexpress the c-*erb*B-2 protooncogene.[2,17–23] Because some tumor cells overexpress p185^{erbB-2}, a dose of AR209 that will kill tumor cells without concomitant significant damage to normal cells is possible. This therapeutic dosage window is particularly important for the treatment of prostatic tumors because normal cells in vital organs, such as the lung, express p185^{erbB-2}. Bronchial epithelial cells express very low levels of p185^{erbB-2}, whereas certain prostatic tumors express excess protein (fig. 1). The presence of high levels of p185^{erbB-2} in tumors has been associated with poor prognosis;[32] therefore, patients with aggressive disease may be particularly good candidates for therapy with AR209. Our data indicate that AR209 is not effective in killing large tumors (fig. 2, *C*); therefore, AR209 probably would not be an effective treatment for localized disease or for total prostate ablation. However, AR209 has proven to be effective in reducing the size of small tumors< 200 mm.[3] This suggests that AR209 may be effective in eliminating or preventing metastatic lesions.

Treatment of PC-3 tumors with a continuous s.c. infusion of AR209 between days 21 and 35 (fig. 2, *D*) resulted in a relatively flat curve during this period of time; however, the tumors did begin to grow upon removal of the osmotic pumps. Data indicate that immunotoxins can accumulate in s.c. xenografts.[33] Sung et al have shown that the distribution for immunotoxins in xenografts is heterogeneous with large spatial separation.[34] They proposed that this discordant distribution was due to impeded infiltration of the tumor. Rippley and Stokes[35] found that an immunotoxin's ability to pene-

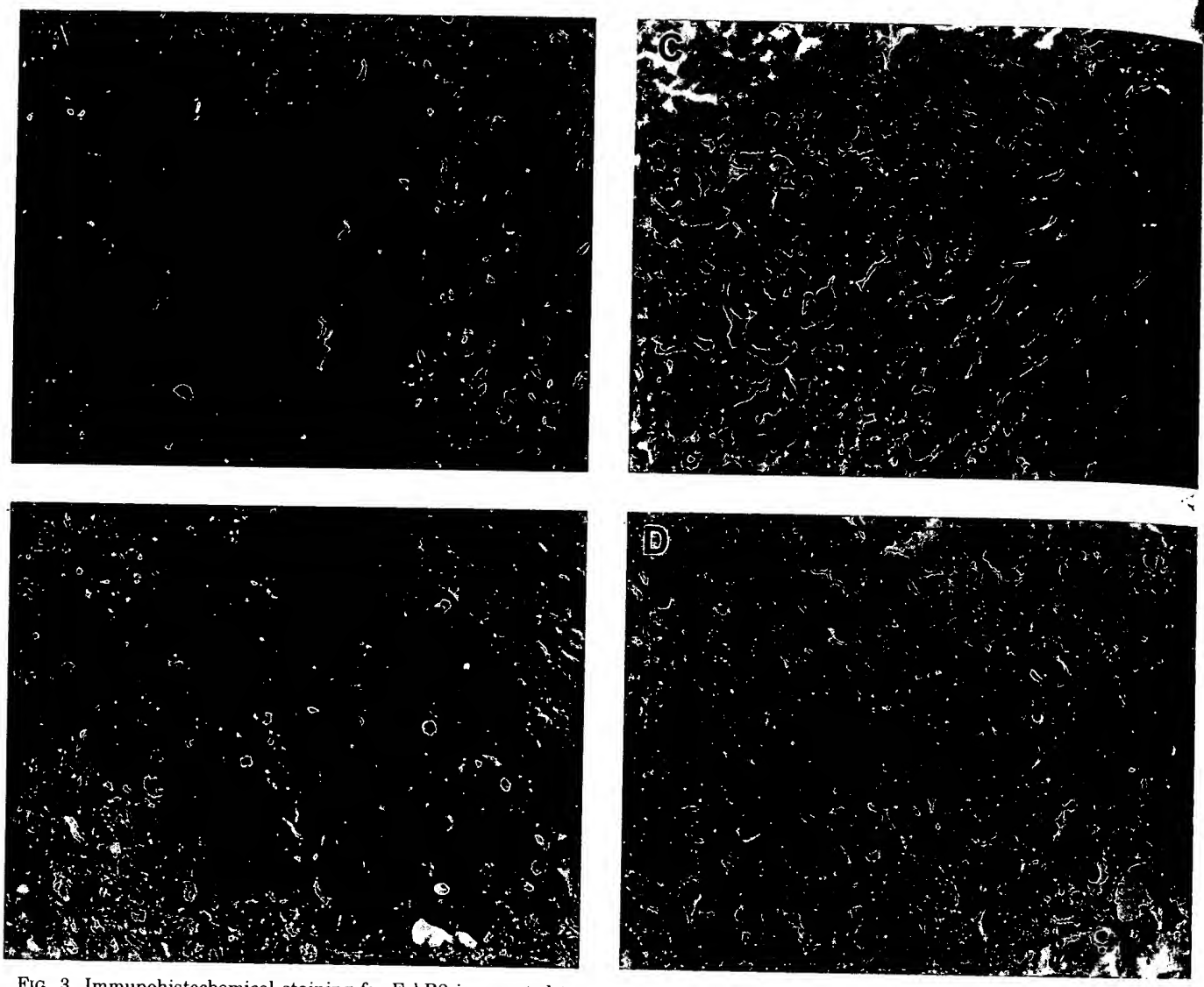

FIG. 3. Immunohistochemical staining for ErbB2 in resected tumor sections. *A*, greater than 75% of DU-145 cells stained positive for ErbB2 before treatment with AR209. *B*, after treatment with AR209 many DU-145 cells remained positive for ErbB2 suggesting that further treatment may have been effective at reducing tumor size. *C*, for PC-3, less than 25% of cells stained positive for ErbB2. *D*, even fewer cells were positive for ErbB2 after treatment with AR209.

trate a tumor was strongly affected by 2 factors: (1) the affinity to which the drug binds to its receptor, or (2) the density of the receptor on the cellular surface. High receptor affinity or increased receptor density reduced permeation of the drug while increasing the peak concentration of intracellular drug. Therefore, maximal drug distribution and optimal therapeutic effect may be mutually exclusive for drugs that require internalization to be effective,[35] as does AR209. Ironically, PC-3 may be a perfect candidate tumor cell line for treatment with AR209. The heterogeneous expression of p185^{erbB-2} may allow deeper penetration into the tumor. DU-145, which has a more homogenous expression pattern for p185^{erbB-2}, was only slightly affected by the continuous infusion of AR209. Because the DU-145 tumors were large, it seems likely that the oncotoxin was unable to penetrate deep into the tumor, because AR209 has the capacity to bind to essentially every cell in the DU-145 tumor. On the other hand, AR209 cannot bind to every cell in the PC-3 mass and probably penetrated deeper into the tumor. It is not clear, however, if this heterogeneous binding would effectively eradicate the tumor. There are data to suggest that not all cells within a tumor need be targeted due to a significant bystander effect.[36,37] However, the effect of AR209 on PC-3 tumors appears to be static rather than extirpative (fig. 2, *D*). This may also be due to the relatively low stability of AR209 after prolonged storage at 37 C.[38] The development of recombinant oncotoxins is a rational approach to drug design. Based on these data, it appears that AR209 may be an effective option for clinical use.

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British Journal of Cancer, (1998) Vol. 78, No. SUPPL. 2, pp. 19

INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY, BIOLOGY, PHYSICS, (1998 Nov 1) 42 (4) 817-22

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EUROPEAN JOURNAL OF CANCER, (SEP 1999) Vol. 35, Supp. [4], pp. 1388

IDrugs, (1999) 2/7 (624-628)

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1/31

Group ch14.18 + GM-CSF neuroblastoma trial had detectable levels of the anti-idiotype Ab. Two distinct anti-idiotype response patterns were evident. Most patients showed anti-idiotype Ab only after exposure to ch14.18. However, occasional patients had detectable levels of anti-idiotype Ab after cytokine therapy, but prior to ch14.18 administration. Decreases in ch14.18 half-life during the second course of treatment corresponded to anti-idiotype production in 3 out of the 4 evaluable patients with a positive anti-idiotype response. A separate assay documented that the anti-idiotype antibody induced in these same 3 patients prevented binding of GD2 by ch14.18. The clinical influence of these distinct anti-idiotype patterns requires further clinical evaluation.

(Proc. ASCO 1997;16:
3622W94 were tested.
up to 7 doses, then m
transient rises in amyl
toms. The following cli
resolved within 24 hour

Table 1: Number

Toxicity	G
nausea	
vomiting	
fever	
abdominal pain	
asthenia	
diarrhea	

A second regimen consi
showed similar toxicity
experienced an allergic-
tachycardia, hypertensic
events were managed by
resolved promptly. Low
responses were detecte
(mean ± sd) at the 30 r
MAb 3622W94 is well
regimens. Phase II trials
3622W94.

*1681

PHASE II TRIAL OF THE BISPECIFIC ANTIBODY MDX-H210 (ANTI-HER2/NEU X ANTI-CD64) COMBINED WITH GM-CSF IN PATIENTS WITH ADVANCED PROSTATE AND RENAL CELL CARCINOMAS THAT EXPRESS HER2/NEU. *N. James, P. Atherton, A. Koletsky, N. Tchekmedyian and R. Curnow. CRC Institute for Cancer Studies, Queen Elizabeth Hospital, Birmingham B15 2TH UK. Intercenter Cancer Research Group, Palm Beach County, FL. Pacific Shores Medical Group, Long Beach, CA. Medarex, Inc., Annandale, NJ.*

We are currently undertaking a phase II study to determine the effects of bispecific antibody MDX-H210 (which combines a recognition site for HER-2/neu with a triggering sequence for the high affinity IgG Fc receptor CD64) combined with GM-CSF in patients with hormone refractory metastatic prostate or locally advanced or metastatic renal cell cancers that express HER2/neu. Other criterion: life expectancy >6 months, and (for prostate cancer) serum PSA >50 or <50 ng/ml and rising. GM-CSF (5 μg/kg/day by subcutaneous injection for 4 days) is followed by MDX-H210 (15 mg/m^2 by intravenous infusion). This is repeated weekly for 3 weeks; each cycle therefore, lasting 18 days. Patients showing response without dose limiting toxicity may repeat the 18 day cycle following a 24 day break. Reported here are results of 5 patients with renal cancer and 18 patients with prostate cancer who have completed at least one cycle. We have seen 2 objective responses in patients with renal cancer: a 52% reduction in the tumor (liver metastases as large as 10 × 12 cm) in one patient and a 49% reduction in the size of a pulmonary metastasis with clearing of non-measurable lesions in another patient. We have seen 5 objective responses (>50% decline in PSA) in patients with prostate cancer: 104 to <0.1, 20 to <0.1, 118 to 11, 872 to 207 and 126 to 58 ng/ml. Quality of life improvements have been noted. Toxicity with GM-CSF was mild (worst NCI-CTC grade 2). Little or no additional toxicity was seen in the majority of patients after infusion of MDX-H210; however, in responders, grade 3 toxicity was seen in both renal cancer patients and 1 prostate cancer patient. We believe this is the first evidence of response to immunotherapy in patients with hormone refractory prostate cancer, and indicates promising potential for MDX-H210 plus GM-CSF in the treatment of patients with renal and prostate cancer.

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page S 343 + S 344

Thanks,

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significantly higher relapse rate compared to ctx ± rtx, but those pts were salvaged by effective ctx.

1384 POSTER DISCUSSION

Radioth rapy f r stage I testicular s min ma – A pr spective trial

J. Classen, H. Schmidberger, R. Souchon, M. Bamberg. *Dep. of Radiooncology, University of Tuebingen, Germany*

Purpose: With high cure rates of 95–98% for radiotherapy (XRT) of stage I (CS I) testicular seminoma it is the aim of modern treatment strategies to reduce the intensity of adjuvant XRT without compromising locoregional tumour control. We therefore conducted a multicenter prospective trial for limited XRT of CS I seminoma with reduced treatment portals and small total doses. Data on the "per protocol" population have been reported previously. We now present updated results including data of those patients with protocol violations.

Method: Patients with histologically proven pure CS I seminoma received adjuvant XRT to the paraaortic lymph nodes only. Treatment portals stretched from the upper border of thoracic vertebra 11 (T11) to the lower border of lumbar vertebra 4. The total dose was 26 Gy in 2 Gy daily fractions. Acute and late side effects of treatment were prospectively scored using the EORTC score.

Results: Between 4/91 and 3/94 721 patients were enrolled for the trial. 670 patients were eligible for an interim analysis in 1/99. 483 patients were treated strictly per protocol (PP), 187 patients showed protocol violations (PV). Mean follow up was 55 months. There have been 24 cases of relapse, 18 (3.7%) in the PP and 6 (3.2%) in the PV group. There was no in-field recurrence. 22/24 patients were salvaged with two cases of disease-realted death. Statistical analysis showed no differences in relapse rate nor survival between the study populations. Acute side effects of adjuvant XRT were moderate.

Conclusions: Limited volume XRT for CS I seminoma yields high cure rates with moderate acute side effects.

1385 POSTER DISCUSSION

$E_{400}P$ in good prognosis advanced seminoma. The Spanish germ-cell cancer group experience

J.A. Arranz, X. García del Muro, J. Gumà, J. Aparicio, R. Salazar, A. Saenz, J. Carles, M. Sánchez, J.R. Germà. *On behalf of the Spanish Germ-Cell Cancer Group, Spain*

Objective: To analyse response, toxicity, time to treatment failure (TTF) and survival (OS) in patients (p) with IGCCG good-prognosis advanced seminoma treated with $E_{400}P$ (cisplatin 25 mg/m^2/d and etoposide 100 mg/m^2/d × 4 d).

Methods: Since 1994 63 p with were included, 48 p with advanced disease at diagnosis (76%) and 15 p who relapsed after stage I (13 p follow-up, 1 p RT and 1 p carboplatin × 2 after orchiectomy). Mean age was 38 y (19–83), Metastatic sites were retroperitoneum (89%), mediastinum (10%), other lymph nodes (19%) and lung (2%). Royal Marsden stages were II: 84%, III: 14% and IV: 2%. Sixteen p (25%) had high BHCG levels, 16 p had LDH > 2 × N, and 61 p (97%) were classified as MIRC good-prognosis (*Fosse et al, Eur J Cancer 33: 1380–87*). Number of cycles administered were 3 (7%), 4 (80%), 5 (10%) or 6 (3%); 3 p are still on treatment.

Results: Grade 3–4 toxicities were anemia (3%), thrombocytopenia (3%), neutropenia (32%), mucositis (3%), neurotoxicity (2%), alopecia (96%) and emesis (4%). Twenty-one p (33%) received prophylactic filgastrim and 53/60 p (85%) received ≥80% of the maximum dose intensity. All p responded (72% CR, 28% residual disease). After a median follow-up of 26 m, treatment failed in 4 p (6%). Failure was defined as viable tumor after CT (1 p), regrowth of a residual mass (0 p), relapse (3 p) or unacceptable toxicity (0 p). These 4 p had MRC good-prognosis and normal BHCG; 3 of them were IIa–b. One p is on 2nd CT, the other 3 p achieved a 2nd CR (1RT, 2CT). One p died after a 2nd relapse. Median TTF and OS have not been reached. Three-year TTF and OS are 92.5% (95%CI: 85.4–99.6%) and 97% (95%CI: 91–100%) respectively. All IIc–IV p are alive and their 3-year TTF is 96%.

Conclusion: In our experience, $E_{400}P$ is a safe regimen for patients with good prognosis advanced seminoma. This regimen could reduce acute and late toxicities observed with the more standard $E_{500}P$ or BEP regimens.

1386 POSTER DISCUSSION

Identificati n of prognostic subgroups in patients (PTS) with po r risk germ cell cancer (GCT): A cart analysis

C. Kollmannsberger[1], C. Nichols[2], C. Meisner[1], J. Beyer[3], A. Harstick[4], J.T. Hartmann[1], L. Kanz[1], C. Bokemeyer[1]. [1]*University of Tuebingen, Germany;* [2]*Health Science University Oregon, United States;* [3]*University of Berlin;* [4]*University of Essen, Germany*

Purpose: Only a few data exist about prognostic criteria within the group of pts who exhibit poor prognostic criteria according to the IGCCCG classification.

Methods: We retrospectively analyzed the data of 332 pts with 'IGCCCG' poor risk GCT using the classification-and-regression-tree model (CART). The following variables were included: primary localization, presence of visceral or lung metastases (met.), presence of an abdominal tumor, number of metastatic sites, levels of β-HCG, AFP and LDH. All patients had been treated with cisplatin/etoposide-based CTx within randomized clinical trials.

Results: Patient characteristics: gonadal/retroperitoneal (G/R) primary tumor 260 pts (78%), mediastinal primary, tumor 72 pts (22%), visceral met. 205 pts (62%), lung met. 247 pts (74%), abdominal tumor 241 pts (73%), elevated AFP, β-HCG or LDH levels 235 (71%), 253 (76%) and 275 (83%) of pts, respectively. Pts with primary, mediastinal disease and lung met. exhibited the worst 3-year PFS (28%), whereas pts with primary G/R disease and without visceral met. showed the longest 3-year PFS (75%). Pts without visceral met and primary G/R tumor had the most favourable 3-year OS (79%). In contrast, pts exhibiting visceral met. from a primary mediastinal tumor displayed the worst 3-year OS (40%).

Conclusion: Different prognostic subgroups can be identified within the group of poor risk GCT. These data may help to estimate detailed individual prognoses and to identify subgroups of high risk pts that may, in turn, be included in new treatment strategies.

1387 POSTER DISCUSSION

Acute and late sequelae in conventionally fractionated and hyperfractionated conformal radiotherapy in prostate cancer. Preliminary evaluation

R. Valdagni[1], C. Italia[1], P. Montanaro[1], A. Lanceni[1], P. Lattuada[1]. [1]*Casa di Cura S. Pio X, Radiation Oncology, Milan, Italy*

Purpose: To evaluate acute and late toxicities in patients affected by prostate cancer treated with conformal radiotherapy using conventional (STD) or pure hyperfractionated (HFX) regimens.

Method: One hundred patients (pts) were treated with 5-field conformal radiotherapy to prostate and seminal vesicles; 85 were evaluable for this analysis. Forty-two pts were treated with STD-CRT at a total dose (ICRU p.p.) of 73.5–75.5 Gy (median: 75.5 Gy; mean: 74.7 Gy); 43 were treated with HFX-CRT at a total dose of 78.3–82 Gy (median: 80.7 Gy; mean: 80.2 Gy). Acute and late toxicities according to RTOG-EORTC criteria were evaluated weekly during CRT, one month after CRT and 3–4 times yearly afterwards.

Results: No significant worsening of acute toxicities was observed using HFX-CRT (grade 2 max. incidence with HFX vs STD: G.I.: 56% vs 62%; G.U.: 33% vs 31%; grade 3 max. incidence with HFX vs STD: G.I.: 0% vs 0%; G.U.: 9% vs 17%). Actuarial probability at 20 months of grade 2 G.U. toxicity was 13% with HFX and 23% with STD, while grade 2 G.I. toxicity was 20% with HFX and 19% with STD. Only one pt, belonging to the STD-CRT group, experienced a grade 3 toxicity (G.U.). Erectile function in pre-radiotehrapy potent patients was maintained at one year in 86% of HFX and 69% of STD pts.

Conclusion: HFX-CRT seems to favourably compare with lower dose STD-CRT with respect to treatment feasibility and acute/late sequelae.

1388 POSTER DISCUSSION

Immunotherapy with the bispecific antibody MDX-H210 (anti-HER2 × anti-CD64) combined with GM-CSF in HER2 positive hormone resistant prostatic cancer

N.D. James[1], P.J. Atherton[1], A.J. Howie[1], S. Tchekmedyian[2], R.T. Curnow[3]. [1]*CRC Institute for Cancer Studies, University of Birmingham, Birmingham, United Kingdom;* [2]*Pacific Shores Medical Group, Long Beach;* [3]*Medarex Inc, Annandale, United States*

Purpose: Treatment of hormone resistant cancer is palliative in nature and new therapies are urgently needed. We report results following treatment with the bispecific antibody MDX-H210 (anti-HER2 × anti-CD64)

plus GM-CSF in patients with HER2-positive, hormone refractory prostate cancer.

Patients and Methods: Patients were treated with GM-CSF 5 μg/kg/day by subcutaneous injection for 4 days plus MDX-H210 15 mg/m^2 by intravenous infusion on day 4, repeated weekly for 6 weeks.

Results: 25 patients entered the trial, 1 received no treatment and 20 were assessable for response. Toxicity was generally NCI-CTG 0–2. There were 2 grade 4 adverse events (nausea and vomiting, spinal cord compression, probably related to disease progression). 7 of 20 (35%) evaluable patients had a partial PSA response (reduction of >50%), ranging from 51% to 99%, of duration 71, 83, 89, 122, 128, 160+ and 184+ days. A further 6 patients experienced minor PSA responses (reduction <50%, >25%) of 41, 89+, 131, 140, 152 and 165 days duration. 5 of 16 (31%) patients with evaluable pain had improvements in pain scores. The PSA relative velocity (rate of change of the natural logarithm of the PSA level) on therapy was compared to the period prior to study entry and decreased in 16/18 (89%) assessable patients. Median duration of follow up was 105+ days (range 21–188 days) with 6 patients continuing on treatment.

Conclusions: The combination of GM-CSF and MDX-H210 is active in hormone refractory prostate carcinoma. Toxicity was generally mild to moderate and mostly manageable on an outpatient basis. Further studies in prostate cancer are indicated.

1389 POSTER DISCUSSION

Effect of high dose Rhenium 186 HEDP with stem cell support on skeletal metastases in prostate cancer

A. Al-Deen[1], V.R. McCready[2], D.P. Dearnaley[1], J. Treleaven[3]. [1]*Institute of Cancer Research, Academic RT, Sutton;* [2]*Royal Marsden NHS Trust, Nuclear Medicine, Sutton;* [3]*Royal Marsden NHS Trust, Haematology Sutton, United Kingdom*

Introduction: Isotope treatment has an established role in the treatment of prostate cancer bone metastases. The activity given is limited by bone marrow suppression. We have explored the use of Rhenium 186 HEDP in a phase I dose escalation protocol using peripheral stem cell support.

Patients and Methods: 14 patients with hormone resistent advanced prostate cancer with skeletal metastases were given activities of 1400 to 3488 MBq of Rhenium 186 HEDP. Seven received activities above 3000 Mbq. Following growth factor stimulation peripheral stem cells were harvested pre-treatment and returned at day 12 post-treatment. Metastases on whole body scans pre-treatment were compared with these on average 10 weeks post-treatment and activity scored as: not visible, decreased, no change, increased.

Results: Treatment was well tolerated and peripheral blood counts recovered to the normal range in all patients. No patients developed clinically significant thrombocytopenia or neutropenia. The total number of metastases (areas of increased uptake on pre-treatment scan) ranged from 10–70 per patient in the >3000 MBq group and 7 to 31 in the <3000 MBq group. The change in appearance of metastases after treatment was documented. Of the 223 metastases identified in the >3000 MBq group 26%, 16%, 13% and 46% were in "not visible", "decreased", "no change" and "increased" categories respectively post-treatment compared to 6%, 7%, 57% and 31% respectively for the 106 metastases in the <3000 MBq group. Compared with the the number of metastases in the pre-therapy examination there were 3% new metastases in the >3000 MBq group and 49% in the <3000 MBq group at the time of the second scintigram. There was no obvious relationship between the number of metastases nor their size and the reponse to therapy.

Conclusion: These results demonstrate that some metastases can be successfully ablated by therapeutic activities of Rhenium 186 and higher activities are more effective.

1390 POSTER

Increase in stage at presentation in prostate cancer: Have thresholds for referral risen?

J. Dawson[1], E. Elves[1], D. Wallace[1]. [1]*University Hospital, Urology, Birmingham, United Kingdom*

Screening and case finding for early prostate cancer has been much debated in the United Kingdom and widely practiced in Europe and North America. The UK has adopted a case finding approach rather than screening. After running a TRUS clinic in a 'Quick Early Diagnostic Unit' for 5 years we were concerned that we were not seeing any increase in low stage disease.

Patients: Over the last three years 785 patients were seen and biopsied. Criteria for being seen have remained unchanged and only patients with a raised PSA (>4 ng/l) or a suspicious rectal exam were seen.

Results: Total referrals, total number of cancers and cancer stage are shown. There was a trend towards increasing PSA and age over the three years, though this did not reach statistical significance.

Variable	1996	1997	1998
Total referrals	274	237	274
Total Cancers (% of total referrals)	99 (36%)	84 (35%)	115 (42%)
Stage			
T1cM0 (% of all cancers)	33 (33%)	27 (32%)	21 (18%)
T2M0 (% of all cancers)	23 (23%)	21 (25%)	29 (24%)
T3–4M0 (% of all cancers)	19 (19%)	20 (24%)	44 (38%)
M1 (% of all cancers)	18 (18%)	11 (13%)	32 (28%)

Conclusion: Despite the increase awareness of prostate cancer among doctors and public, the case finding approach adopted in our practice has not seen any increase in early disease. This is unexpected and cause for concern. A more aggressive approach to the detection of prostate cancer within the UK is required.

1391 POSTER

Metastatic transitional cell carcinoma: Evaluation of prognostic factors and change in prognosis during the last 20 years

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Purpose: To investigate patients with metastatic urothelial cancer and propose the most appropriate combination of prognostic variables describing the outcome, and to analyse changes in overall survival during the past two decades.

Methods: Between 1992 and 1997, a total of 156 patients with recurrent locally advanced disease (non-resectable, radio-resistant) and/or metastatic transitional cell carcinoma of the urothelial tract were included in a protocol evaluating prognostic factors and pattern of metastases.

Results: Distant metastases were diagnosed in 86% with lymph nodes (57%) and bones (40%) as the most frequent localizations. Liver metastases were found in 21%. Median survival after recurrence was 5.8 months. Multivariate analysis showed that good performance status (PS), normal alkaline phosphatase (AP), absence of liver metastases and chemotherapy were independent prognostic factors for long survival. Comparison was made with 240 patients treated in the period from 1976–1992. A significant increase in survival in the present period was found.

Conclusion: PS, AP and liver metastases are the major important prognostic factors. Stage migration and increased use and efficacy of chemotherapy has resulted in increased survival in metastatic urothelial cancer.

1392 POSTER

Length of follow-up influences biochemical control rates after treatment for prostate cancer

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To determine whether biochemical control (bNED) rates following treatment for prostate cancer are dependent on the length of post-treatment follow-up (f/u), we reviewed 437 patients with clinically localized prostate cancer treated with conformal radiotherapy without neoadjuvant androgen deprivation (AD). Biochemical failure was defined as three consecutive PSA increases or an increase large enough to prompt salvage AD. The date of failure was back-projected to the midpoint between the PSA nadir and the first PSA increase (or between the nadir and the initiation of salvage therapy). The analysis was performed by censoring patients with longer f/u in a step-wise fashion, thus creating smaller subgroups with shorter f/u. Subgroup 1 (N = 191) and Subgroup 2 (N = 273) were defined to include those patients followed for up to 2 years and up to 3 years, respectively. No significant differences were seen in pre-treatment prognostic factors among the three groups. The 2-year bNED of Subgroup 1 (median f/u = 1.1 years), Subgroup 2 (median f/u = 1.5 years), and the original population (median f/u = 2.5 years) were 86.3%, 77.4%, and 73.4% (p = 0.05). No differences in clinical recurrence rates were seen between any of the three groups. In conclusion, bNED rates are highly dependent on the length of f/u. This appears to result from the back-projection of failure dates, which is a component of commonly used bNED definitions. This has important implications

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An EORTC crossover trial comparing single-agent Taxol® (T) and doxorubicin (D) as first- and second-line chemotherapy (CT) in advanced breast cancer (ABC). *R. Paridaens, P. Bruning, J. Klijn, T. Gamucci, L. Biganzoli, A. Van Vreckem, G. Hoctin Boes, M. Piccart on behalf of EORTC-IDBBC/ECSG participating centres.*

From August 1993 until May 1996 a total of 331 anthracycline-naïve patients (pts) were randomised to receive either T (200 mg/m^2, 3h inf. q 21 d) or D (75 mg/m^2 q 21 d) as first-line CT for ABC. Crossover was mandatory in case of progression occurring within the first seven cycles of first-line CT, but optional for progression occurring later. Time to progression (TTP) is the primary end point of the study. Response rate (RR) and quality of life are also being evaluated. To date, 294 pts are evaluable for toxicity and 271 for response to first-line CT. Pts are evenly distributed in terms of prognostic factors except for soft tissue as dominant site of disease: 12% in arm D vs 6% in T. As expected, the toxicity profile of the 2 agents is different (data given as % of pts).

CTC grade	Neutropenia (G4)	Neutropenic fever	Stomatitis (G3-4)	Neurosensory /Arthralgia (G3)
T	39	6	1	8/5
D	82	18	14	-/-

The overall RR, according to an intent to treat analysis, is currently 36%, for first-line CT (T + D combined) and 24% for second-line treatment. Sufficient events will have occurred by April 97 to compare TTP and RR of the 2 agents given as first-line CT for ABC.

*540

A randomized phase III study of Taxotere® (T) versus doxorubicin (D) in patients (pts) with metastatic breast cancer (MBC) who have failed an alkylating containing regimen: preliminary results. *S. Chan[1], K. Friedrichs[2], D. Noël[3], R. Duarte[4], D. Vorobiof[5], T. Pintel[6], L. Yelle[7], M. Alaki[8], M. Murawsky[9], A. Riva[4] - [1]City Hospital (Nottingham), [2]Eppendorf University (Hamburg), [3]Hôpital Sacré-Coeur (Montreal), [4]NCI (Bogota), [5]Sandton Oncology Center (Johannesburg), [6]Country Hospital (Gyor), [7]Hôpital Notre-Dame (Montreal), [8]Rhône-Poulenc Rorer (Antony).*

T as single agent has been shown to be highly active in MBC. This phase III trial compared T at 100 mg/m^2 as a 1 hour IV q^3 wks to D at 75 mg/m^2 q^3 wks. As of November 1996, the accrual is complete: 312 patients were randomized, 156 in each group. Preliminary results are presented on 102 pts on T and 98 pts on D. The main pts characteristics are comparable between T and D. Median age was 48 years (range 31-74); median Karnofsky index 90% (range 60-100); prior chemotherapy: adjuvant only in 49%, advanced disease only in 45% and both in 6%; 79% of pts had visceral disease including 45% with liver metastases. Out of 198 pts evaluable for safety (2 pts not treated), the main grade (gr) 3 and 4 adverse events by pts in T *vs* D respectively were: neutropenia 93% *vs* 93%; infections 3% *vs* 6%; febrile neutropenia 16% *vs* 13%; nausea 4% *vs* 18%; vomiting 3% *vs* 14%; stomatitis 5% *vs* 14%; diarrhea 10% *vs* 1%; asthenia 13% *vs* 11%; septic death in 1 pt in each group; 2 pts withdrew due to fluid retention in T and 6 pts due to cardiac function toxicity in D.

Intent to treat analysis	Taxotere (n = 102)	Doxorubicin (n = 98)
Response rate	47%	26.5%
Complete response	10%	3%
Median TTP	29 wks	21 wks
Median no of cycles (range)	7 (1-10)	5 (1-7)
Median RDI (range)	0.98 (0.05-1.07)	0.95 (0.49-1.05)

At equally myelosuppressive doses T is showing a higher anti-tumour activity than D. Final results will be presented at the meeting.

*541

Correlation of circulating c-erb B-2 extracellular domain (Her-2) with clinical outcome in patients with metastatic breast cancer (MBC). *M.J. Stender, D. Neuberg, W. Wood, G. Sledge. Indianapolis, IN, Boston, MA, Atlanta, GA.*

Retrospective analyses of patients (pts) receiving chemotherapy for MBC have suggested that Her-2 expression is correlated with response to chemotherapy, disease free interval, and overall survival. We examined plasma samples from 319 pts enrolled in ECOG 1193, a phase III trial in pts receiving Taxol-based chemotherapy for MBC. Measurements (in duplicate) were made using an enzyme immunoassay for quantitative measurement of human Her-2 protein in plasma (provided by Chiron Diagnostics), with positive values defined as > 30 u/ml. Of the original 319 samples available, 30 samples were from pts ineligible from the parent study, and 9 lacked sufficient identification. The remaining 280 are included in this analysis. One hundred nine pts initially received single agent Taxol, 107 Adriamycin and Taxol, and 64 initially received Adriamycin alone and crossed over to Taxol at progression.

Fifty of 216 pts (23%) receiving Taxol at induction are classified as Her-2 positive. There was no association between Her-2 positivity and objective response to chemotherapy (p=0.51). Of 64 pts evaluated for response to Taxol on crossover from Adriamycin, 11 (17%) were Her-2 positive. Again, there was no association between Her-2 positivity and objective response (p= 1.0). Overall survival was measured from the date of induction randomization to date of death or last date known alive. Thirty-four of 61 (56%) Her-2 positive pts have died; median survival is 17.7 months. Sixty of 219 (31%) Her-2 negative patients have died; Median survival is estimated at 30.2 months. Her-2 positive pts have a statistically worse survival (p=0.0008). We conclude that while there appears to be no association between Her-2 status and response to therapy, circulating Her-2 positivity strongly predicts for poorer overall survival in MBC.